Amendment to the claims

Claim 17 is amended to more succinctly define the method of the invention and to address the Examiner's rejections under §§ 102 and 103, as discussed in more detail below, by more thoroughly distinguish the claimed method from the methods of the prior art. Support for the addition of step (c) of amended claim 17 is on lines 10 to 17 of Example 6 on page 41 of the specification and in claim 83 of the originally filed application.

Claim 85 is amended to include fibroblast growth factor among the grouping of preferred growth factors for the proliferation of multipotent CNS stem cells. Support for this amendment is on page 23, line 19. At the Examiner's request, data can be provided that demonstrates the proliferative effect fibroblast growth factor has on multipotent neural stem cells.

Claim 87 is amended to address the Examiner's § 112 rejection as detailed below.

Claim 88 is amended to more succinctly define the method by which multipotent stem cells are continuously perpetuated *in vitro*. Support for the amendment is as noted above in Example 6 and in claim 83 of the originally filed application.

The sentence bridging pages 40 and 41 of the specification supports the amendment to claim 89.

Claims 90 and 91 are amended to more succinctly define the claimed methods.

Claim 93 is amended to define a method of proliferating a multipotent neural stem cell whereby the progeny of the stem cell produce a clonally-derived neurosphere. A neurosphere is a cluster of cells generated from a single stem cell and hence, the cells of the

neurosphere are clonally-derived — see description of Figure 1 on page 15, lines 25-27 of the application.

Claim 94 is added to further define a method of passaging the progeny of a multipotent stem cell when the progeny grows in the form of a neurosphere. The term "passaging" is a well-known term used in the field of cell and tissue culture. The method described in the beginning of Example 6 on page 41 is a description of cells being passaged.

Rejections under 35 U.S.C. § 101

Applicants acknowledge the provisional rejection of claims 17, 18, 20 and 85-93 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over the claims of copending applications Ser. Nos. 07/961,813, 07/967,622 and 08/010,829. Applicants will respond to this provisional rejection when there is an indication of allowable subject matter.

Applicants wish to note that U.S. Ser. No. 07/967,622 has been abandoned in favor of continuation application U.S. Ser. No. 08/221,655.

Applicants also wish to draw the Examiner's attention to copending application U.S. Ser. No. 08/149,508 which claims priority from the captioned-application.

Rejections under 35 U.S.C. § 112

The specification stands objected to under § 112, first paragraph. The Examiner states on page 5, lines 9-10 of Paper No. 13 that applicants have "failed to provide evidence showing

proliferation of neural tissue from adults or juveniles". A Rule 132 Declaration of Dr. Brent A. Reynolds is filed concurrently with this Preliminary Amendment. In paragraphs 3-4 of the Rule 132 Declaration, is a discussion of Applicants' publication in Science which supports the assertion that adult tissue can be obtained which contains at least one multipotent stem cell which can be proliferated *in vitro* to produce progeny to give rise to multiple types of differentiated neural cells (i.e. glial cells and neurons).

On p. 5, lines 24-28 of Paper No. 13 (in the discussion of the rejections under §112) the Examiner states "regarding Applicants' further arguments that claim 92 is not rendered obvious by the prior art, note that the independent claim, claim 17, does not claim adult tissue and the combination of references renders obvious the claimed invention for reasons as set forth below". Applicants are not certain what is meant by this statement and request clarification. In any event, Applicants' response to the § 103 rejections of claim 92 are discussed in detail below.

In the paragraph bridging pages 5 and 6 of Paper No. 13, the Examiner comments that "Applicants have failed to provide evidence that adult tissues obtained from the adult mouse and human would give rise to multipotent stem cells and that the stem cells could be proliferated *in vitro*". The data set forth in paragraphs 4 and 5 of the Rule 132 Declaration show that neural tissue obtained from adult mice gives rise to multipotent stem cells which can be proliferated *in vitro*. The data set forth in paragraphs 7-9 and the photographs of Appendix B of the Rule 132 Declaration show that neural tissue obtained from an adult human gives rise to multipotent stem cells which can be proliferated *in vitro*.

On page 6, lines 3-5 of Paper No. 13, the Examiner states that "Applicants have failed to disclose evidence that amphiregulin would have the claimed results, which is the proliferation of multipotent stem cells *in vitro*". Paragraph 10 of the Rule 132 Declaration provides evidence that multipotent stem cells can be isolated from mammalian neural tissue and proliferated *in vitro* in response to treatment with amphiregulin.

Regarding claim 88, the Examiner states that "Applicants have failed to provide evidence that the stem cells may be proliferated *in vitro* without limit" (Paper No. 13, p. 6, lines 6-7). As pointed out in paragraph 11 of the Rule 132 Declaration, using the methods of the claimed invention, neural stem cells have been passaged over 30 times over the course of 8 months. After 30 passages, the cells maintain their proliferative ability and the ability to produce progeny that differentiate into neurons and glia. This evidences that the cells proliferated by the methods of claim 88 are stem cells. Attached as Exhibit A is a discussion on the characteristics of stem cells taken from Molecular Biology of the Cell [Alberts *et al.*, Garland Publishing, Inc. (1983)]. This supports the assertion that stem cells are undifferentiated cells that are capable of dividing without limit. According to Potten and Loeffler, the defining features of a stem cell are that it is an undifferentiated cell capable of proliferation, self-maintenance (i.e. capable of dividing without limit) and the production of a large number of differentiated, functional progeny [Development 110:1001-1020 (1990); attached as Exhibit B]. Also attached as Exhibit C is a review article on stem cells by Hall and Watt which states that "stem cells, by definition, have an extensive capacity for self-

renewal that extends throughout the life of the organism and so is, essentially, unlimited" (p. 628, end of col.1) In addition, this article illustrates other stem cell characteristics.

In all events, for purposes of rendering the claim 88 more precise, it has been amended to more succinctly define the method by which the progeny of a multipotent stem cell can be continually perpetuated. New claim 94 is added to define the method of passaging the progeny when they are in the form of neurospheres in suspension. This ability to isolate, proliferate and continually passage undifferentiated neural stem cells was unknown prior to the present invention.

Regarding claim 92, the support for claiming the proliferation of stem cells derived from juvenile or adult tissue is provided as set forth in the Rule 132 Declaration.

Regarding claim 93, the claim has been amended to define a method wherein the progeny of the multipotent stem cell grow in the form of a clonally-derived neurosphere. Support for this amendment in the specification is as noted above.

Claims 17, 18, 20 and 85-93 stand rejected under 35 U.S.C. § 112, first paragraph for the reasons set forth in the objection to the specification. It is believed that this rejection is overcome in view of the comments made above, the evidence provided herewith which supports these claims, and further in view of the amendments to the claims.

Claim 93 stands rejected under 35 U.S.C. § 112, second paragraph. Amended claim 93 does no longer contains the language objected to by the Examiner. Therefore, it is believed that the rejection to this claim is overcome.

Claim 87 stands rejected under § 112, second paragraph. The Examiner states that "the phrase 'preexposed to serum' is vague and unclear since when the preexposure takes place is not apparent" (Paper No. 13, p. 7, lines 5-8). The claim is amended to replace the term "preexposed" with "exposed". As amended, it is clear that the multipotent stem cell is proliferated in a defined culture medium (i.e. a medium that does not contain serum). Additionally, the multipotent stem cell is not exposed to serum *in vitro*. Thus, while the cell may have been exposed to serum *in vivo*, once the cell is dissociated from the mammalian neural tissue it is not exposed to serum *in vitro* and is proliferated in a defined culture medium. The Examiner states that "it is not known in the art to expose cells to serum before putting the cells into a medium" (Paper No. 13, p. 7, lines 8-9). However, Cattaneo *et al.* (cited by the Examiner) disclose dissociating cells from rat striata and exposing the cells to 10% fetal calf serum prior to culturing the cells in defined culture medium (see caption to Figure 1). Thus, claim 87 further distinguishes the methods of the present invention from the methods described by Cattaneo *et al.*

The Examiner also states that "the culture medium usually contains serum" (Paper No. 13, p. 7, lines 9-10). The term "defined culture medium" is a term of art which means that the molecular constituents of the medium are known. A serum-containing medium is not defined because serum is a complex biological substance of which not all the components are known and which varies amongst individuals. Therefore, the entire molecular make-up of serum is never completely defined. The significance of defined medium is described in the McGraw-Hill

Encyclopedia of Science & Technology, pages 389-390 attached hereto as Exhibit D. In addition to the advantages set forth in column 1 of page 390, it is advantageous to use a defined culture medium because serum alters the response of stem cells to growth factors in undefined ways. Additionally, if the cells are to be transplanted into a patient, it is desirable to know the exact make-up of the medium used to culture the cells (e.g. serum may contain undetected pathogens).

Rejections under 35 U.S.C. § 102

Claim 17 remains rejected under § 102(a) as being anticipated by Cattaneo. For a reference to be anticipatory, every element of the claimed invention must be disclosed in the reference. The Examiner states that "the cell of Cattaneo is a multipotent stem cell since the cell was capable of at least two pathways, proliferation of the stem cell phenotype, or differentiation into neurons" (page 7, lines 17-19 of Paper No. 13). The term "multipotent" is an art-recognized term which is equivalent to the term "pluripotent". The discussion of the properties of stem cells in Exhibit A explains that "those stem cells that give rise to only one type of **differentiated** cell are called *unipotent*; those that give rise to more than one type are called *pluripotent*" (p. 913, 2nd full paragraph; italics in original, bold added). Therefore, because the cells described by Cattaneo *et al.* gave rise to only one type of differentiated cell (neurons), the cells were unipotent.

Claim 17 has been further amended to state that the multipotent neural stem cell, that is cultured using the claimed method, is capable of producing progeny that are capable of

differentiating into neurons and glia. The claim is also amended to include an additional step, the passaging of the progeny of the multipotent neural stem cell.

Cattaneo *et al.* do not describe a culture of multipotent neural stem cells. The cells of their cultures do not exhibit self-maintenance (a hallmark feature of a stem cell). In addition, the cells described in this reference only differentiate into neurons as evidenced by neurofilament staining. Cattaneo *et al.* fail to disclose or suggest a culture of cells that is capable of differentiating into cell types other than neurons. Thus from this reference, one of ordinary skill in the art is provided no guidance on how to attain a culture of multipotent neural stem cells (i.e. cells capable of self-maintenance or unlimited proliferation and giving rise to more than one differentiated cell type). While Cattaneo *et al.*, at best, demonstrate a cell culture of unipotent neuronal progenitor cells capable of proliferation for a limited period of time (9 days, see Fig. 1) before differentiating into one cell type (i.e. neurons), such does not suggest the possibility of attaining a culture of proliferating multipotent neural stem cells, the progeny of which are capable of differentiating into neurons and glia.

In view of the amendment to claim 17 and for the above reasons, it is maintained that Cattaneo *et al.* fail to anticipate or render obvious the method of claim 17.

Claims 17 and 85-90 stand rejected under § 102(a) as being anticipated by Anchan (Neuron). Claim 17 has been amended to include an additional step (c): the passaging of the progeny of the multipotent stem cell. Anchan *et al.* are not concerned with the proliferation of multipotent stem cells and the passaging of the

progeny of multipotent stem cells. As noted in paragraph 12 of the Rule 132 Declaration, the methods of the prior art focus on the limited proliferative ability of committed progenitor cells and the subsequent differentiation of these cells. The cells studied by Anchan *et al.* adhere to tissue culture substrates and begin to flatten (note the description of the cells bridging pages 923 and 924; trypsin-EDTA is required to get the cells to lift off the substrate for counting purposes). The nonadherent cells in the Anchan cultures, which may or may not have included multipotent stem cells, are largely ignored (see p. 925, middle of column 1). These cells were merely counted. There is no suggestion that the nonadherent cells are multipotent CNS stem cells which can be continuously proliferated upon passaging.

The adherent cells which were studied by Anchan *et al.* are capable of limited amounts of proliferation prior to differentiation. On page 925, bottom of the first column, they report that "...cells, plated at an initial density of 10^5 increased to almost... 3.5×10^5 cells per well at the end of this same period".

Anchan *et al.* do not show that a **single cell**, grown according to their methods, is **multipotent**, capable of generating neurons and glia (astrocytes and oligodendrocytes). Instead, they only show that an aggregate of cells (10^5 cells per well) can multiply and differentiate into a variety of retinal cell types. It is possible that there were a number of different types of unipotent committed progenitor cells present in the retinal neuroepithelial tissue placed into each well which proliferated and differentiated.

Anchan *et al.* summarize the work others have done regarding the effects of EGF on various classes of CNS cells, stating

that it has been found that "EGF is mitogenic for astrocytes and oligodendrocytes; however, no mitogenic effects were reported for neuronal progenitors" (see p. 933, bottom paragraph). Therefore, the Anchan reference would lead one away from using EGF to try and proliferate multipotent neural stem cells, the progeny of which are capable of differentiating into glial cells ***and neurons***, from sources of CNS tissue other than retinal neuroepithelium.

Claim 87 requires that the culture medium used in the proliferation of the multipotent stem cell be defined. The medium used by Anchan *et al.* contained serum and thus was not defined (see discussion of Anchan's experimental procedures on p. 934; see Exhibit A for definition of "defined culture medium"). The advantages of using a defined culture medium for the proliferation of cells which are to be used for therapeutic purposes are noted above.

As amended, claim 88 calls for an additional passaging of the progeny of a multipotent stem cell. Anchan *et al.* do not disclose or suggest a method for the passaging of stem cells.

Regarding claim 89, the Examiner states that "Anchan discloses proliferation of cells in a suspension culture..." (Paper No. 13, p. 9, lines 15-16). However, as noted above, Anchan *et al.* only disclosed the limited proliferative ability of adherent cells. The only discussion of nonadherent cells is on p. 925, column 1, wherein it is noted that "between 10% and 20% of the total cells plated" were nonadherent. These cells were merely counted. There is no discussion as to the identity or characteristics of these cells, or whether these cells proliferated *in vitro*.

Regarding claim 90, the cells of Anchan *et al.* were both proliferated and differentiated while plated (see p. 934, column 2). They do not use plating the cells as a way of ***inducing*** differentiation of the proliferated cells as set forth in claim 90. Furthermore, the cells of Anchan *et al.* are primary cells obtained from dissociated tissue and have not been passaged at least once prior to differentiation. The culture technique of the present invention allows proliferated cells to be passaged multiple times prior to differentiation, resulting in large numbers of differentiated cells.

In view of the amendment to claim 17, and for the above reasons, it is believed that the rejection of claims 17, and 85-90 under § 102(a) as being anticipated by Anchan (Neuron) is overcome.

Claims 17 and 89 stand rejected under § 102(b) as being anticipated by Temple. As amended, claim 17 calls for the passaging of the progeny of the multipotent stem cell to further proliferate the progeny. Temple does not describe a method whereby proliferated cells are passaged to induce further proliferation. Thus, the reference cannot anticipate the claimed method.

Temple provides no evidence that her culture techniques caused proliferation of a multipotent stem cell. In the middle of column 2 on p. 473, it is stated that a few rare cells in the culture "...***may be*** multipotential stem cells which can generate several cell types and can renew themselves..." (emphasis added). However, no evidence is provided that this is the case. This observation is made simply because the cells did not stain for neurofilament or

GFAP and therefore were unlikely to be differentiated neurons or astrocytes. These cells may have been committed progenitor cells that had not expressed detectable levels of neuronal or astrocyte antigens. No attempt was made to isolate and proliferate these cells or characterize them as stem cells (i.e. demonstration of self-maintenance and generation of a large number of progeny). The cells were not stained for nestin to determine whether they were undifferentiated CNS cells.

One of the larger clones observed by Temple is reported to have 34 cells after 10 days consisting of 12 neuron-like cells, 20 GFAP astrocytes and 2 cells that did not stain for either marker. Suggesting that such a small number of clonally derived cells are stem cell-derived would be inaccurate as Temple did not demonstrate that any of these cells exhibit stem cell properties (i.e. there was no demonstration that the cells were capable of self-maintenance and the production of a large number of differentiated, functional progeny; see Exhibit B]. The generation of 34 progeny from a single cell can be accomplished by a progenitor cell (this would not be considered to be a large number of cells generated from a single progenitor cell obtained from a tissue [the brain] which contains billions of cells). This inability of Temple to identify any stem cells may be a result of at least 2 scenarios: 1) the culture conditions do not promote stem cell proliferation or 2) stem cells were not present in any of the wells. In the first scenario, the culture conditions used by Temple and the Applicants' claimed methods vary substantially (i.e. use of serum and live cell conditioned medium by Temple). In addition, by using Applicants' claimed methods, a typical neurosphere will have several hundred

cells after only 6-7 days *in vitro* (see paragraph 11 of the Rule 132 Declaration). In the second scenario, it is noted that less than 0.1% of cells obtained from embryonic day 14 rat striatum are multipotent stem cells (see paragraph 12 of the Rule 132 Declaration). Thus, it is very probable that by culturing only a few hand-selected cells dissociated from rat brain septal tissue (see caption of Fig. 1 on p. 472), Temple did not select any stem cells that may have been present in the dissociated tissue (theoretically, one would need to plate at least a thousand cells to isolate a stem cell).

Regarding claim 89, the Examiner states that "Temple discloses a cellular suspension of cells and does not plate out the cells onto a substrate..." (Paper No. 13, p. 10, lines 3-4). However, Temple says nothing about proliferating cells in suspension. Also, it is not readily apparent that Temple "inherently discloses a method wherein the cell is proliferated in suspension" as stated by the Examiner. The cells of Temple were cultured in poly-L-lysine coated wells (see caption to Fig. 1), a substrate commonly used to induce cell adhesion and differentiation (see sentence bridging pages 24 and 25 of Applicants' specification). Additionally, from Temple's Figures, it is apparent that the cells were adhered.

In view of the amendment to claim 17 and for the above reasons, the methods of claims 17 and 89 are not anticipated by the Temple reference.

Claims 17, 18, 85-88, 90 and 93 stand rejected under § 102(a) or (b) as being anticipated by Reynolds, Tetzlaff and Weiss (Abstract 474.2). The Reynolds *et al.* Abstract is the applicants'

own work and was published in October 1990, less than one year prior to the priority date of the application. Although W. Tetzlaff is listed as an author of the abstract, the criteria for authorship of a scientific journal is not necessarily the same as inventorship under the patent laws. Declarations under § 715.01(c) of the MPEP of Drs. Weiss and Reynolds are attached hereto which set forth rationale as to why W. Tetzlaff is not an inventor of the claimed subject matter [see *In re Katz* 215 USPQ 14 (CCPA 1982)] Accordingly, the rejections of claims 17, 18, 85-88, 90 and 93 should be withdrawn.

Claims 17, 18, 85, 86, 87, 88 and 90 stand rejected under § 102(f) in view of the Reynolds *et al.* reference that includes Dr. Tetzlaff as an author. As requested by the Examiner, declarations are provided as to why Dr. Tetzlaff is not listed as an inventor of the application. Accordingly, it is believed that this rejection is overcome.

Rejections under 35 U.S.C. § 103

Claims 91 and 92 stand rejected under § 103 as being unpatentable over Anchan (Neuron) in view of Reh *et al.* The Examiner states that "Reh discloses culturing isolated cells *in vitro* to the aggregate stage and then dissociating the cells to cause differentiation" (Paper No. 13, p. 11, lines 26-28). Reh does not disclose that individual cells *in vitro* are proliferated to form aggregates which are then dissociated to induce differentiation. The methods outlined in the Reh paper (p. 4179) clearly indicate that the retinal tissue is dissociated, plated and allowed to differentiate. They do not ***proliferate*** individual cells in suspension.

They found differences between cells which occurred singly after dissociation and those which were incompletely dissociated (i.e. which they plated in small aggregates), but no mention is made anywhere of culturing cells which, due to proliferation, ***formed*** aggregates which were then dissociated to induce differentiation.

Regarding claim 92, the Examiner states that "it would have been obvious.. to apply the techniques of Anchan to tissue from adult and juveniles in order to obtain proliferation of multipotent stem cells in view of the teachings of Anchan that in larval amphibians removal of the retina induces a dramatic regeneration of in (sic) the remaining marginal neuroepithelial cells" (Paper No. 13, p. 12, lines 4-6). The knowledge that marginal neuroepithelial cells are known to regenerate in ***larval amphibians*** does not provide a reasonable expectation of success that multipotent stem cells can be isolated and proliferated from ***juvenile*** or ***adult mammalian*** (see claim 17) neural tissue. To the contrary, at the time the invention was made, it was commonly believed by those even highly skilled in the art that neural stem cells simply do not exist in the adult central nervous system. Attached as Exhibit E is an article from The New York Times, (March 27, 1992), which demonstrates that researchers in the field of neurobiology were of the belief, at the time of the invention, that neural stem cells were not found in the mammalian CNS. Note the comment made by Dr. Emmanuel DiCicco-Bloom: "It wasn't thought possible that you would find this (stem cells that can proliferate and produce neurons) in the mature mammalian brain". The comments made by various researchers in the field of neuroscience which are quoted in this article provide

compelling secondary evidence of the unobvious nature of the claimed invention.

For the foregoing reasons, the combination of Reh and Anchan fail to render claims 91 and 92 obvious. Additionally, neither reference is concerned with a method of proliferating multipotent stem cells and ***passaging*** the progeny of the multipotent stem cells to proliferate said progeny (as required by step c of claim 17, from which claims 91 and 92 depend). At best, these references teach the limited proliferation of retinal progenitor cells.

Claims 85-90 stand rejected under § 103 as being unpatentable over Cattaneo in view of Anchan (Neuron). It is maintained that both of these references, as well as the other references cited by the Examiner, are concerned only with primary cultures of neural cells that exhibit minimal proliferation prior to differentiation. The combination of these references fails to suggest a method for the proliferation of a multipotent stem cell. The arguments made above with respect to the teachings of Cattaneo *et al.* are reasserted here. Cattaneo *et al.* fail to disclose the proliferation of a multipotent neural stem cell which can proliferate to produce progeny capable of differentiating into neurons and glia. Cattaneo *et al.* disclose the minimal proliferation of unipotent neuronal progenitor cells.

Claim 17, from which claims 85-90 depend, has been amended to include an additional step (c), which ***very clearly*** distinguishes Applicants' methods from that of the prior art. The prior art cited by the Examiner provides no suggestion of a method by which multipotent stem cells can be proliferated and passaged. Moreover, the prior art fails to provide a reasonable expectation of

success that a multipotent neural stem cell (capable of self renewal) can be isolated from mammalian tissue and proliferated *in vitro* to produce progeny capable of differentiating into neurons and glia.

Regarding claim 87, the above arguments are reasserted here: Anchan *et al.* did not culture their cells in defined medium.

Regarding claim 88, the above arguments are reasserted here: Anchan *et al.* did not demonstrate the proliferation of a multipotent stem cell *in vitro*. Moreover, claim 88 has been amended to define a method by which multipotent neural stem cells are proliferated to produce progeny which are then passaged, proliferated again and passaged again. The prior art fails to suggest a method of the continuous proliferation and passaging of multipotent neural stem cells.

Regarding claim 89, it is reasserted, for the reasons discussed under the § 102 rejection, that Anchan does not disclose proliferation of cells in a suspension culture.

Regarding claim 90, it is reasserted that Anchan *et al.* do not ***induce*** differentiation by plating cells onto a fixed substrate. The cells of Anchan were both proliferated (albeit minimally) and differentiated while adhered to the substrate.

The Examiner states that "Cattaneo provides the motivation to combine the references..." because it states "that other members of the NGF family might promote both the proliferation of neuronal precursors and the survival/differentiation of neurons...". For a proper § 103 rejection, not only must there be a suggestion in the prior art to make the combination, but there must also be a reasonable expectation of success [see In re Vaeck, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991)]. In any event, Cattaneo does not

provide the requisite motivation to combine the references. While Cattaneo *et al.* state that "NGF and other members of the NGF family ***might*** promote both the proliferation of neuronal precursors and the survival/differentiation of neurons derived from these precursors" (p. 765, last sentence; emphasis added), Cattaneo *et al.* teach away from using other growth factors. Cattaneo *et al.* state: "Furthermore, combination of NGF or bFGF with other growth factors did not produce any effect on cell number (data not shown)" (p. 763, 2nd col., last sentence). This statement, together with the comment in the Anchan paper that EGF does not promote the proliferation of neuronal precursors, would have discouraged one of ordinary skill in the art from using other growth factors to promote proliferation of CNS progenitor cells.

Additional Rejection under 35 U.S.C. § 102

Claims 17, 85, 86, and 89 stand rejected under § 102(b) as being anticipated by Anchan *et al.* (Abstract 308 in J. Cell Biol). Claim 17, as amended, requires the proliferation of a multipotent stem cell and the passaging of the progeny of the stem cell. Anchan *et al.* do not disclose either of these features of claim 17. The Examiner states that "Anchan inherently discloses that the germinal epithelial cells were multipotent stem cells since the cells either proliferated or differentiated, thus demonstrating that the cells had multiple fates" (Paper No. 13, p. 14, lines 3-6). As noted above, the term "multipotent" refers to an undifferentiated cell that can give rise to more than one type of differentiated cell. The Anchan abstract only discloses that undifferentiated cells

proliferated and gave rise to neurons. They do not disclose the presence of other cell types in their cultures.

Additionally, references relied upon for the purposes of § 102 must be enabling, thus placing the allegedly disclosed matter in the possession of the public [Akzo N.V. v. Int'l Trade Commission, 1 USPQ 2d 1241, 1245 (CAFC 1986)]. Anchan *et al.* is not an enabling reference because it provides no details of culture techniques. Thus, the rejection under § 102 based on this reference is improper.

Additional Rejections under 35 U.S.C. § 103

Claims 18, 20, 87, and 90-93 stand rejected under § 103 as being unpatentable over Anchan (abstract) in view of Anchan (Neuron) and Reh.

The arguments raised above with respect to all three of these references are reasserted here:

1. Anchan (abstract) does not disclose proliferation of multipotent stem cells. They only disclose cells that differentiated into neurons.

2. Anchan (Neuron) do not demonstrate the proliferation of a multipotent stem cell. Additionally, the reference would lead one away from using EGF to try and proliferate multipotent neural stem cells, the progeny of which are capable of differentiating into glial cells ***and neurons***, from sources of CNS tissue other than retinal neuroepithelium.

3. Contrary to the Examiner's assertion, Reh *et al.* do not disclose culturing isolated cells *in vitro* to the aggregate stage and then dissociating the cells to cause differentiation. The aggregates

of Reh *et al.* result when the tissue that was cultured was incompletely dissociated.

Regarding claim 87, the above arguments are reasserted here: Anchan *et al.* did not culture their cells in defined medium.

Regarding claim 90, it is reasserted that Anchan *et al.* do not ***induce*** differentiation by plating cells onto a fixed substrate. The cells of Anchan were both proliferated (albeit minimally) and differentiated while adhered to the substrate.

Regarding claim 91, the comments with respect to the teachings of Reh *et al.* are reasserted here.

Regarding claim 92, it is maintained that, at the time the invention was made, it was wholly unexpected and very surprising that multipotent neural stem cells could be isolated from adult tissue and proliferated *in vitro* (see Appendix E).

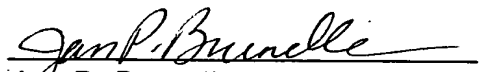
Regarding claim 93, Anchan (abstract) provides does not disclose or suggest a method by which multipotent stem cells can be proliferated and passaged.

None of these references disclose the proliferation of a multipotent neural stem cell that produces progeny capable of differentiation into neurons and glia. Additionally, claim 17 has been amended to include an additional step: the passaging of the progeny of the multipotent stem cell. The combination of these references fails to suggest a method for the continuous proliferation of multipotent neural stem cells.

In view of the foregoing, it is respectfully submitted that the present claims are in condition for allowance. Early notice of such allowance is solicited.

Respectfully submitted,

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT



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